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Chromatographic Separations

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Overview

Pharmaceutical analysis is traditionally defined as analytical chemistry dealing with drugs both as bulk drug substances and as pharmaceutical products (formulations). However, in academia, as well as in the pharmaceutical industry, other branches of analytical chemistry are also involved, viz. bioanalytical chemistry, drug metabolism studies and analytical biotechnology. The development of drugs in the pharmaceutical industry is a long-term process, often taking more than a decade from the start of a research project to the appearance of a drug on the market. That process involves several decision points, such as the choice of the candidate drug after the preclinical screening phase, the investigational new drug (IND) application before testing the compound for the first time in man, and finally the new drug application (NDA) which summarizes the data obtained from all the studies needed for marketing approval of the drug as a medicine. In all these steps, especially the IND and NDA, the amount of data generated is enormous. Analytical chemists take part in many of the studies that constitute this documentation. Substance quality and its specifications are based on substance analysis, and that knowledge is later used for quality control during full-scale production. Product analysis involves dealing with the various formulations and starts after the IND has been approved. The results from such work lead to specifications that form the basis for the quality control of the product. For both substances and formulations there is an increasing interest in the introduction of process analytical chemistry.

Biomolecules, i.e. macromolecules such as proteins or hormones, either produced by isolation from biological sources or by means of biotechnology, must also be subjected to careful analytical control. Thus whilst the analytical tasks required for biomolecules are somewhat different from those of ordinary pharmaceuticals when it comes to regulation and documentation of their quality and properties they definitely belong to the same group.

There are a number of regulations that have to be followed in the development of pharmaceuticals as well as in their production. Regulatory approval is required prior to the IND and before marketing is licensed (NDA). Today clinical trials also undergo scrutiny by the authorities.

An important part of the development process is safety evaluation, primarily the toxicology tests, which run from 6 to 24 months in different species. During this time bioanalytical studies are performed as well as control of the formulations used in the tests. After approval for marketing, the authorities exercise control of products on the market and require post production stability data. Public interest in the quality of drugs is also reflected in the compilation of substance monographs in compendia that are known as pharmacopoeias. In addition to collections of substance monographs these pharmacopoeias contain general analytical methods and some also contain monographic requirements on the formulation of the substances.

This article provides an overview of mainly substance and product analysis (traditional pharmaceutical analysis), as used in the pharmaceutical industry. The support of other branches of analytical chemistry will be mentioned.

Bulk Drug and Pharmaceutical Products

Common Features

Identity testing Identity testing is used to verify that the drug substance is what it is stated to be or that the formulation contains the correct compounds. Infrared (IR) spectra are used quite extensively in industry, whereas the pharmacopoeias often have a set of alternative tests. These can be colour reactions, melting point of a compound or derivative, optical

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rotation values or ultraviolet (UV) spectral data such as maximum wavelength and absorptivity. Today chromatographic data are also used to support identity tests.

Impurities Impurities or degradates require separation methods and are usually studied at the level from 0.1–2% (purity patterns) or 0.1–5% (stability profiles). This means that the analytes have to be quantified in up to a 1000-fold excess of the major compound. In practice qualitative work is performed at still lower levels. This sometimes creates problems in the chromatographic methods as minute amounts of related substances may be hidden under the peak of the drug itself. This is the background for the interest in peak purity tests. With the advent of diode-array detection in liquid chromatography (LC) compounds with different chromophores may be differentiated, either through spectral comparison or by absorbance ratioing at selected wavelengths. However, a peak impurity present at below 1% may be difficult to detect. The use of mass spectral data for the verification of peak purity is still better and has been practised in gas chromatography–mass spectrometry (GC-MS). However, peak purity tests are inferior to the use of complementary separation systems.

Selected analytes Selected analytes sometimes have to be analysed at ppm levels. Typical examples are aromatic amines, nitrosamines, reactive intermediates left from the synthesis, or certain solvent residues, i.e. all components that are known to be noxious and thus must be controlled separately.

Compendial analysis Pharmacopoeias are the official collections of drug standards. They all include requirements for drug substances but only a few have monographs for products (formulations). Harmonization efforts between the compendia for the three big markets, Europe, Japan and the US, have recently been started. There is an agreement now that the major pharmacopoeias are in principle intended for the pharmaceutical industry and the authorities and not for community pharmacies.

Bulk Drug Analysis

Physicochemical characterization Physicochemical characterization yields a number of important parameters that can be used in the control of the quality of a substance. Typical properties are melting point and other thermal data, acid-base behaviour with pK_a values, redox potentials, polymorphism, solubility and spectral information.

Purity tests Purity tests are in particular focused on related substances such as homologues, analogues, by-products from the synthesis or degradates. Enantiomeric purity has been a more common test since the early 1980s. Chromatography has revolutionized our ability to determine substance purity and is currently the most important check, giving essentially the finger-print of a synthesis. An example of a separation of some potential impurities that have been added to a metoprolol sample is given in **Figure 1**. For qualitative studies LC-MS is beginning to establish its role as the primary on-line analytical tool for the elucidation of unknown structures among the impurities. From a toxicological point of view the impurity profile of the substance batch used in safety studies should form a reference for the full-scale production material. This means that in later batches impurities in amounts that deviate from those found in the batches used for toxicology should be avoided. The high standard and good reproducibility required of the purity profile is clearly evident from that perspective. There are some other tests that also contribute to the general impression of the quality of a substance, i.e. tests for protolytic impurities, content of chloride, sulfated ash or residue upon ignition that gives the inorganic content. These tests reflect the performance of the purification process in general, but their importance will probably diminish in the future. For inorganic ionic analytes the older methods can be replaced by ion chromatography.

Heavy metals and arsenic Heavy metals are routinely determined, often with one or other form of sulfide precipitation. These tests are performed from the viewpoint of safety and the general limits (1–30 $\mu g\ mL^{-1}$ (g^{-1})) are now more often related to the dose. For metals such as mercury, lead, cadmium or nickel, atomic absorption spectrometry or other instrumental methods are often prescribed. Copper and other transition metals can act as catalysts in certain degradation reactions and thus require special attention. Surprisingly, arsenic tests that were important at the beginning of this century are still considered necessary.

Potency assay Common for all bulk drugs is an assay of potency. This can be an aqueous or nonaqueous titration based on protolytic properties or on some other property. Many compounds lack functional groups suitable for titration and here chromatographic methods (LC in particular) are often used. However, titrations are preferable as their precision is, in general, superior.

Biomolecules Biomolecules such as proteins represent a special type of bulk drug. Depending on the

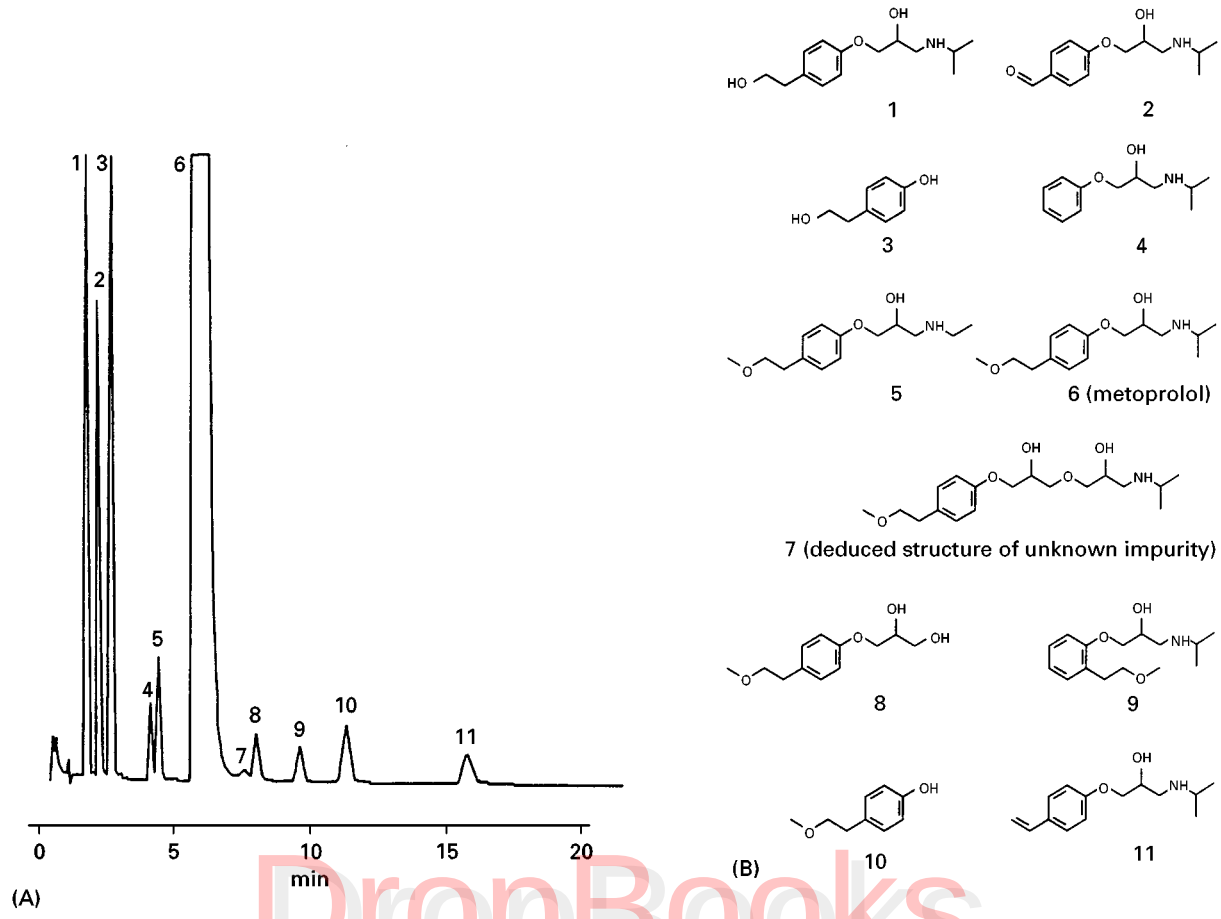

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Figure 1 (A) Separation by LC of metoprolol and some of its potential impurities that have been added in amounts form 0.01 to 0.4% to a pure sample. Peak 6, Metoprolol; peak 7, an unknown purity. Peaks 1–11, see structures in (B). Column: 125 mm long and 4 mm i.d. filled with 5 μm C-8 particles (Li Chrospher RP-Select B). Mobile phase: Acetonitrile, 17% in ammonium phosphate buffer 0.05 mol L^{-1}, pH 3.2. Detection at 280 nm. (With thanks to Lars A. Svensson, Astra Hässle, Sweden.)

source, various chromatographic tests are used to show the absence of contaminating proteins remaining from the purification process. The separation methods used have a more biochemical character and differ from those for compounds of synthetic origin. Immunochemical techniques are very often used as complements. Typical tests include separation of dimers, trimers etc. from the biomolecule itself. Molecular size determination by size-exclusion chromatography is common and will probably be supplemented by mass spectrometry in the future.

Biotechnological products have some advantages over products from human or animal origin with respect to the risk of transferred diseases, but they have some special requirements. In particular, when parenteral use is intended, the absence of host proteins has to be guaranteed as well as DNA residues from the vector used for expression. Such matters often need the attention of specialist laboratories.

Bioassay Many biomolecules have, over the years, been assayed using methods where biological activity in an animal, organ or receptor is assessed, i.e. a bioassay. The traditional opinion has been that such bioassays cannot be replaced by physicochemical methods as the latter do not reflect the biological activity. Bioassays, no matter how well characterized they are, have certain disadvantages with respect to precision, time and cost compared to instrumental methods. The pharmaceutical industry has therefore been able to show that, for several of its biotechnological products, e.g. insulin and human growth hormone, chromatographic methods (LC in particular) can give the same information. Moreover, proteins from different species can be chromatographically separated and degradation products well quantified. This is not possible in quantitative bioassays where only the sum of activities is obtained. A similar paradigm shift has also taken place for antibiotics, where LC methods give information not

available from microbiological assays. However, it should be noted that for many biomolecules there are, as yet, no alternatives to bioassay.

Excipients

There are a great number of materials that are used to transform a substance from an active compound to a medicine useful for a patient in a therapeutic situation. These compounds, or excipients, are becoming more and more important in the construction of modern drug delivery systems. Many excipients are macromolecules and have been used for decades in traditional remedies. With modern drug delivery systems the old requirements may not always fit those necessary for the technologically advanced products of today. This has become increasingly evident in recent years. Moreover, the requirements on excipients in the various pharmacopoeias are not always consistent with each other and are often rather vague. This has been recognized at an international level and efforts at harmonization are proceeding. Polymeric excipients are generally characterized by some average physicochemical property such as viscosity. Studies of the distribution of relative molecular mass are rarely performed on such excipients due to the lack of suitable methods. The majority of the excipients used today, however, have relative molecular masses in the same range as the active compounds. The tests for safety and purity are similar to those of the drugs.

Excipients could usefully be classified or tested according to their properties at three levels, viz. molecular, particular and bulk properties. Those are tested for by the manufacturer of a dosage form. It is not clear which of those properties should be covered by the official compendia. Testing of functionality, i.e. at particulate or bulk level, does not seem to be possible yet. Typical tests are bulk density, specific surface area, flowability and particle size distribution. However, the standardization of methodology in compendia, without specification limits, would probably be of help for both vendor and buyer. As excipients are becoming more and more complex, their analytical characterization will be more important. Interesting opportunities lie ahead, particularly with macromolecular separation, MS and spectrometric methods such as near-infrared (near-IR) spectrometry.

Pharmaceutical Products

A medicine is much more than simply a drug substance, and huge efforts are put into the development of biopharmaceutically optimized drug delivery systems. Analytical chemists contribute to that process by analysing the experimental formulations with respect to various properties such as homogeneity, content, stability and release of the active agent in dissolution testing but also through bioanalysis to create data for *in vitro – in vivo* correlations.

Solid dosage forms Solid dosage forms, e.g. tablets and capsules, are by far the most common for several reasons. The production of relevant doses is easy to accomplish, and scale-up is usually a standard technological process. All divided dosage forms have strict requirements for uniformity of content, i.e. a statistical sampling of the batch should show a uniform distribution of the active component. This requirement is especially important for units with very small amounts of the active component, i.e. from a few μg per dose to 50 mg. This has often led to automated analytical methods to cope with the large number of samples.

The pharmacokinetic performance of a drug influences the contruction of a formulation. This has nowadays led to a dominance of drug delivery systems that provide a controlled or modified release of the drugs, defined as extended or delayed release. Release-controlling polymers are used to build up a barrier that prevents immediate release of a compound. In this way high peak plasma concentrations of drugs are avoided and usually only one dose per day is necessary. The characteristic properties of the formula are evaluated in *in vivo* tests where blood samples are analysed often by extremely sensitive bioanalytical methods.

However, for routine quality control it is usual to rely on *in vitro* models, which obviously have to be correlated with the *in vivo* data. Dissolution testing has been standardized in the pharmacopoeias for a long time with respect to release media, apparatus and other conditions. However, in modified release formulations the prescribed conditions might have to be changed. This is the responsibility of the analytical chemist, whilst still having the routine testing conditions in mind, i.e. quality control (QC) methods should also be practically feasible.

Parenterals Parenterals are dosage forms intended for injection into the body. Water is normally used as the solvent. The special tests for parenterals include sterility and absence of particles as well as endotoxins that can give fever reactions. The old test that was performed on rabbits is nowadays often replaced by a test based on the reaction of endotoxins with a lysate from *Limulus amoebocyte* (LAL-test) that is less time-consuming and more exact. Sterility testing is complicated from a sampling point of view because of the random appearance of microbial contaminants. A thorough in-process validation is the best way to

ensure that the products are sterile. In large volume parenterals' requirements on limits for particle contamination have created a need to analyse for particles down to the size of a few micrometres, usually by light-blocking or by conductivity techniques. For compounds which are sparingly soluble in water other solvents or co-solvents can be used creating problems of quite a different kind for both formulator and analyst, such as evaluation of precipitation phenomena and interactions with packaging materials.

Other dosage forms Other dosage forms are needed for topical administration such as creams, ointments and patches. The latter should usually deliver the drug over an extended period of time and thus require reliable *in vitro* release testing and as a consequence also *in vivo* data.

Sometimes other routes of administration are necessary. Thus suppositories are used for rectal delivery and sprays for the nasal route. Sublingual delivery can be advantageous, e.g. for nitrate esters. For asthma the use of inhalators has increased considerably as reliable hi-tech delivery systems have been developed. Here the uniformity of the inhaled dose (usually a few μg), as well as the narrowness of the particle size distribution, must be safeguarded.

The analytical problems of these dosage forms have to do both with the type of excipients used and their characterization and quality as well as the function of the delivery system.

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Toxicological formulations Toxicological formulations appear in the early project work in short-term toxicology tests performed before testing in humans (pre IND) and later on during long-term carcinogenicity studies before approaching the authorities for marketing approval (pre NDA). The reason for mentioning them here is that at this early stage a full understanding of the properties of a drug is not always available, and most important, the animal feed into which the drug may be blended is a very difficult matrix in which to analyse a drug. Yet, the analyst has to determine the homogeneity and the stability of such formulated animal feeds during the use of the material. Here the rules of Good Laboratory Practice (GLP) are emphasized with careful validation of analytical methods.

Packaging materials Packaging materials are also part of the medicine presented to the patient. They are usually polymers that have properties chosen to give drug the product protection during storage and handling. Usually multilayer materials are used, as in blister packages for tablets, with some layers being a barrier towards moisture penetration.

Plastic bottles are becoming more common now. For many products, particularly parenterals, there is a risk of interaction between the polymeric material and the active component, especially if the latter has a lipophilic character. Rubber stoppers in parenterals are likewise prone to trap organic molecules from, and also leak stabilizers into the solution. Biomolecules in solution may adsorb onto these surfaces and this may be especially significant if the amounts in solution are minute. From these comments it is clear that stability testing must also be performed in the consumer package under appropriate conditions of temperature, humidity and light.

Stability studies Stability studies constitute a major task for the analytical chemical laboratory. The aims of initial physicochemical studies and investigations of incompatibilities, i.e. preformulation studies, are to identify the weak points of a compound, in order to avoid vulnerable conditions in the formulation. An example of an incompatibility reaction in a formulation containing three active ingredients and stored under accelerated conditions is given in **Figure 2**. The structure of the degradate was elucidated with electrospray LC-MS and verified as shown in **Figure 3**. Heat, moisture, oxygen and light may all influence stability.

Tests have to be performed under standardized conditions with respect to temperature and humidity. Accelerated tests can be used to isolate the weak points, but in the documentation submitted to the authorities long-term stability data under normal conditions are required. In these studies stability indicating assays are important and usually LC is the method of choice as the measurement of the drug content should not be disturbed by interfering components. Early degradation is, however, more reliably monitored through the degradates as the precision in the determination of a degradate is less demanding than the measurement of the corresponding decrease in the parent compound.

Full-scale production Full-scale production is the final step in the research process. Scaling up from the pharmaceutical development laboratory via the pilot plant to full-scale production is not without problems. This is one of the reasons for the requirement by the authorities that stability studies should be performed and reported for the first three production batches. The transfer (scaling up) can be facilitated by thorough process controls that give an understanding of those parameters which must be controlled. As pharmaceutical formulations are more complicated today than ever before it is clear that process analytical chemistry will play an important role in the

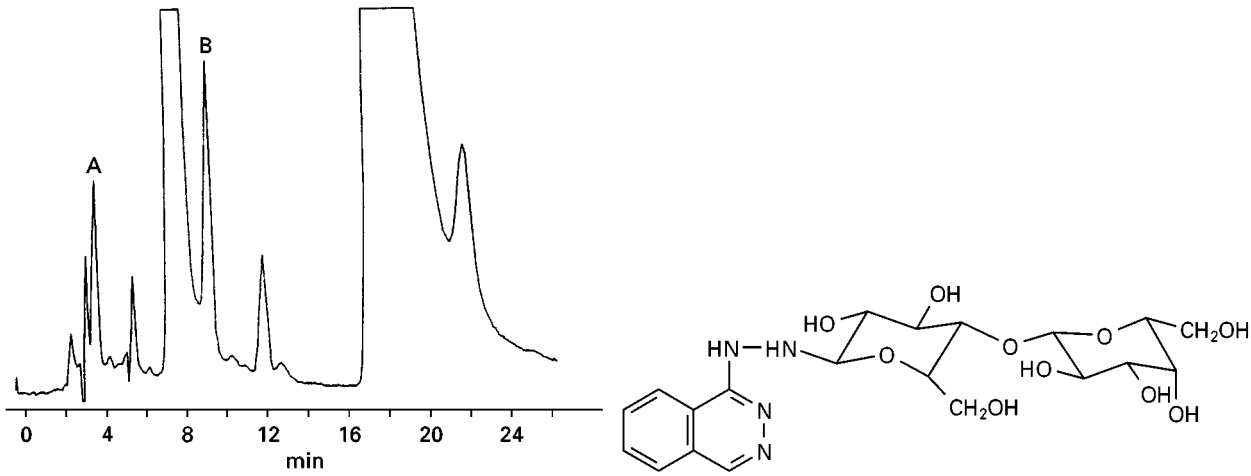

Figure 2 Separation by LC of degradates from a formulation containing hydralazine. After storage at accelerated conditions components A and B started to grow the peaks. They were both the result of an incompatibility with lactose. The structure of B was elucidated by LC-MS as shown in Figure 3. (With thanks to Rose-Marie Janson, Astra Hässle, Sweden.)

future. In this way it will hopefully be possible to control the process by feedback reactions before severe deviations occur. Noninvasive techniques such as near-IR and ultrasonic methods provide interesting possibilities in this context.

Specifications and Quality Control (QC)

Specifications

The quality requirements of a substance in bulk and those of a pharmaceutical formulation are compiled in specifications. In these documents the requirements on the various quality parameters are given as minimum or maximum limits or ranges. Formulations often have requirements on technical properties such as dissolution rate, disintegration and hardness for tablets. All those requirements are the result of comprehensive studies in the R&D phase, where the knowledge of the properties of the drug is gathered, resulting in the optimized formulation of the active component. The analytical and technical test methods that are linked to the specifications will have evolved during the R&D process and can, at the NDA stage, be transferred to QC laboratories.

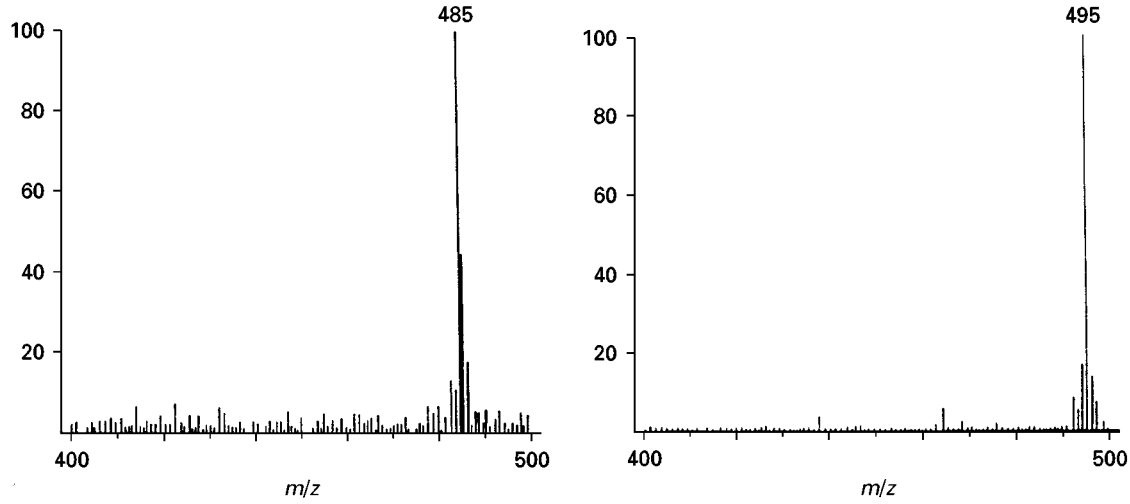

Figure 3 Mass spectra of degraded B in Figure 2. Electrospray LC-MS was used with a 0.5 m fused silica capillary column, i.d. 250 μm. The packing material was 5 μm C-18 Chromasil and the mobile phase was 60% acetonitrile in 5 mmol L^{-1} ammonium acetate with a flow rate of 1.5 μL min^{-1}. The left-hand panel mass spectrum was obtained with the mobile phase consisting of water and the right-hand panel with the use of heavy water (deuterium oxide) instead. The mass number difference is due to nine hydrogen atoms exchanged with deuterium plus the ion charge H^+ being changed to D^+. (With thanks to Karl-Erik Karlsson, Astra Hässle, Sweden.)

It is important to bear in mind that what is used in clinical trials should be reproduced in full-scale production. Of special importance is the particular batch, or so-called biobatch, which is studied *in vivo* and compared with *in vitro* properties as in dissolution testing to establish a correlation *in vivo – in vitro*. This batch is of critical importance for the future and its documentation has to be thorough. The bioavailability, as documented in the biobatch, is the foundation for the coming market presentation. Equally important are the substance batches used in safety studies, and requirements on the knowledge of their quality are very exacting. Also, there is a distinction between release and check specifications. The requirements at the release usually are somewhat tighter than those at a control performed any time during the entire lifetime of a medicine (check specification). In order to be aware at an early stage of deviations from the intended range of a quality parameter in a process the industry works with internal specifications that are tighter than the external ones.

Reference Substances

Most methods require some form of chemical reference substances (CRS), which have already been characterized in an IND more thoroughly than in normal specifications, for example, by adding thermo analytical data and spectrometric data for structural evidence, e.g. infrared, nuclear magnetic resonance and ultraviolet. Compendial methods have official CRS, which are available for customers all over the world. It is important to remember that a reference substance can be used for different purposes, not all of them requiring extensive testing. So a CRS for identity testing is less demanding from a purity point of view. For daily work a less expensive working standard can be calibrated vs. a CRS.

Quality Control (QC)

Full-scale production is checked in the QC laboratories according to the specifications and test methods approved by the authorities. Decisions by QC management cannot be overruled by any person in the organization, which puts a particular onus on the competence and judgement of the person in charge of QC. In addition to the chemical and biological tests that comprise QC the organization can introduce preventive measures to avoid quality impairment. This activity is defined as integrated or total QC and is further outlined in Good Manufacturing Practice (GMP) (see Regulatory Aspects). It cannot be emphasized enough that chemical control at the end of a process can never replace high standards in the process itself. This is sometimes so evident that release in certain cases can be given based on the documentation and control in the process steps. This is called parametric release. Parametric release has been accepted by the authorities in those cases where end control does not fully reflect failures in the production. This has been most evident in many biotechnological processes, where the absence of host proteins, DNA residues or virus particles has been approved through a thorough validation procedure where the purification step is challenged. The analytical methodology is not adequate in this situation at the end control.

Process analytical chemistry may, in the near future, play a similar role in showing that a process does not run beyond prescribed limits. This not only gives better quality in products, but also fewer failures and thus reduced costs. Verification of identity is required at several stages during the process, not only at the end. This can be done in many ways and it is important to remember that the sum of the tests performed during a process also contributes to that verification. Biotechnological products are a special case in that the identity of the recombinant protein with that of the native one has to be established. Thus, it is important that the correct order of the sequence of amino acids is verified. Peptide mapping provides one way of showing this by comparing the chromatographic pattern of peptide fragments obtained after enzymatic cleavage. Capillary electrophoresis (CE) complements LC in this role. In this area there are new and interesting possibilities with mass spectrometric techniques that allow molecular ions to be determined up to and above 200 kDa.

Regulatory Aspects

Good Manufacturing Practice (GMP) was mentioned above and has been a cornerstone of pharmaceutical production and control since the 1970s. These regulations state clearly what has to be done to safeguard quality from the beginning of the production process to the end, viz. documentation, staff qualifications, standard of facilities, technical standards, handling of material, labelling, etc. Control guidelines are mentioned but not in the same detail as in Good Laboratory Practice (GLP). These rules state that all documentation of analytical methods should fulfil certain performance criteria, that instruments should have maintenance records and that their performance and those of the method should be documented. Every analyst should in principle document an analytical method and its aims.

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Validation of Analytical Methods

Validation of an analytical method establishes in laboratory studies that the performance characteristics of the method meet the requirements for the intended application, thus the method does what it is expected to do. In the *United States Pharmacopeia* (*USP*) the following items are listed: precision, accuracy, limit of detection, limit of quantification, selectivity, range, linearity and ruggedness. Of these, accuracy is probably the most difficult to document or obtain, at least for solid formulations. This has to do with the fact that recovery experiments are difficult to design in such a way that they resemble the process conditions. The reactions there can create interactions which are not obtained in an experiment where the analyte has only been mixed or spiked to the sample.

Selectivity is another factor in the validation process that is much discussed. Many authorities require a selective method in a product release specification and mean that a chromatographic procedure has to be carried out even if a simple UV method is free from interferences. Here cost effectiveness should be the guide and analysts should therefore use their scientific arguments to justify using the simpler method. Validation procedures are equally important in the documentation of bioanalytical methods.

International harmonization Harmonization efforts are now being made at an international level. Of special importance is the possibility of having the same guidelines worldwide on how stability studies should be performed and reported. The same is true of guidelines on how to study impurities, especially where the limits should be set and at what level identification is needed. Similar efforts are seen for dissolution testing and how to correlate *in vivo* and *in vitro* data. The question of bioavailability and bioequivalence is an important one and a common view on these issues would be welcome, even if recommendations on how to perform a bioanalytical method will probably never appear in a pharmacopoeia. In recent years the interest in chiral drugs has increased and we can now also see an interest in harmonization for these compounds.

Emerging Techniques in Pharmaceutical Analysis

Analytical chemistry is one of the disciplines most frequently involved in the R&D work performed in the pharmaceutical industry. This makes the industry very analysis intensive, which explains the high level of interest in testing new methods in order to get further information. It is also clear that this interest in

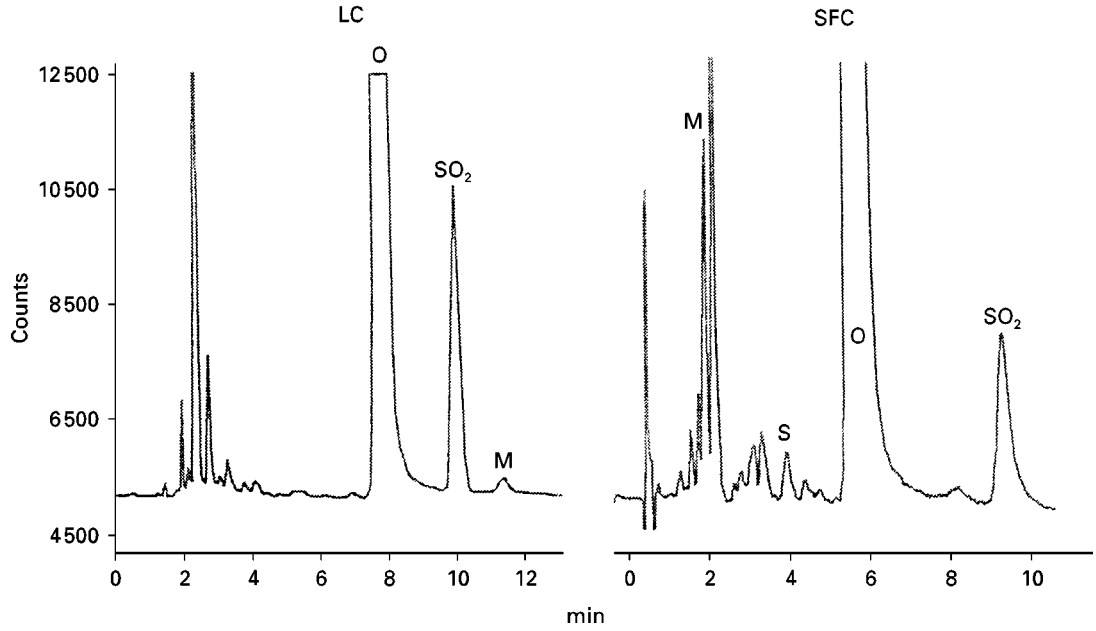

Figure 4 Separation by LC and SFC of omeprazole and some related compounds. Conditions for LC: column 100 mm long and 4.6 mm i.d. filled with 3 μm C-18 (Chrompak Microsphere). Mobile phase: acetonitrile, 26%, in phosphate buffer 0.01 mol L^{-1} and pH 7.4 containing tetrabutylammonium 1.0 mmol L^{-1}. Flow rate: 1.0 mL min^{-1}. Conditions for SFC: column 125 mm long and 4 mm i.d. with 5 μm Li Chrosorb NH_2. Mobile phase: carbon dioxide 2.0 mL min^{-1} with methanol containing 1% triethylamine at 120 μL min^{-1}. Temperature: 40°C. Pressure: 175 bar. Test compounds: 0 = omeprazole (a sulfoxide); S = reduced form, a sulfide; SO_2 = oxidized form, a sulfone, and M = two isomers of *N*-methylated omeprazole (only separated by SFC). (With thanks to Olle Gyllenhaal and Svante Johansson, Astra Hässle, Sweden.)

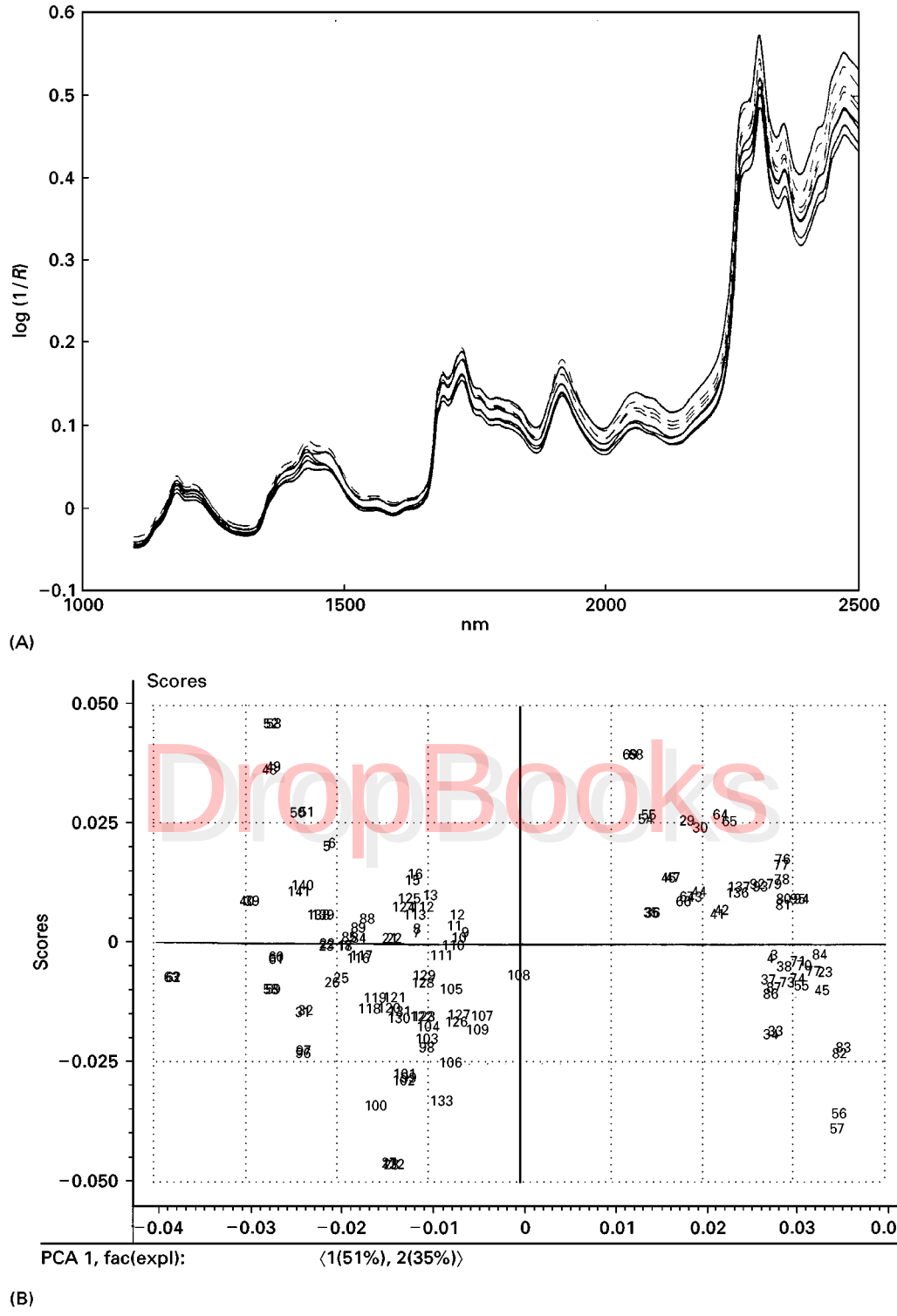

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Figure 5 (A) Reflectance spectra in the near-IR of a number of ethyl celluloses obtained form two vendors. (B) Principal component projection of the spectra in (A) after multiplicative signal correction. The left cluster is from vendor 1 and the right one from vendor 2. (With thanks to Mats Josefson, Astra Hässle, Sweden.)

learning, and using the latest techniques, stems from a desire to obtain reliable information more quickly, or to obtain complementary data.

The research process is a long-term commitment and requires high standards in the results from the very beginning. The quality of the results relies

on competent analytical chemists, but also on the availability of good instrumentation, which is often evident in the laboratories of the research intensive pharmaceutical industry. Separation techniques, especially LC, have had an enormous impact on pharmaceutical analysis. The combination of LC with MS has further extended the possibilities of the techniques as qualitative data can very often be obtained on-line. Can we foresee a similar development in years to come? The development of new techniques usually proceeds in a stepwise fashion and, at present, mass spectrometric techniques are taking a giant step towards the analysis of macromolecules in a reliable way. This revolutionary process has just begun to show promising results with relative molecular masses of more than 200 kDa being determined using time of flight drift tubes. Whether or not these instrumental possibilities can be combined on-line with separation methods, especially CE, remains to be seen. These MS extensions will have great impact on biotechnology products and macromelecular compounds used as excipients.

Another area where rapid development is taking place is Raman spectrometry. New technology has opened interesting possibilities for this old technique. Many opportunities lie ahead both in regular and in process analysis.

Separation methods will see continued growth, particularly the capillary techniques. Capillary chromatography is particularly useful when expensive mobile phases are used. A good example of LC-MS using packed fused silica columns 250 μm in diameter is given in Figure 3. Here heavy water was used interchangeably with an aqueous phase. Hydrogens bound to heteroatoms (O, N, S) were then replaced with deuterium, which was easily revealed in the mass spectrometer. This tells the analyst how many labile hydrogens the molecule has (in the case of the example this was 10). For macromolecules up to particles, the use of field flow fractionation (FFF) techniques can be expected to increase. Complementary information to LC will come from CE and supercritical-fluid chromatography (SFC). The latter technique will probably find a place in product analysis, especially in the packed column version, where polar compounds can be analysed. Packed column SFC is an interesting complement to LC, showing more rapid and efficient separations. An example is given in **Figure 4**, where omeprazole and some related compounds are separated. The sulfide form is eluted at about 25 min in LC, i.e. twice the retention in SFC. Capillary electrophoresis has recently become a useful separation method in the pharmaceutical industry, not least for biotechnological applications.

Chemometric methods will have an impact on many types of technique, particularly in areas where properties which are not easily measurable (such as taste, texture, etc.) have to be correlated with physicochemical parameters. In this context, near-IR has started to gain prominence within the pharmaceutical industry. An example from the analysis of a number of cellulose ether samples with reflective near-IR is shown in **Figure 5**. The individual spectra do not show much in the way of differences. The spectra were corrected for different light penetration depths by multiplicative signal correction (MSC). Then the entire spectra were projected by principal components analysis (PCA) as points on a plane. The samples could be grouped into two clusters in this plane, one for each vendor. The calibration of this technique is entirely dependent on multivariate analysis or chemometrics and this combination will grow in use. In the same way process analytical chemistry is going to proceed based on multivariate data. Many noninvasive approaches rely on near-IR and multivariate calibration. New approaches combined with techniques that will stand harsh process conditions will also be introduced. Clearly, in the future new principles of measurement may also be necessary and the analytical chemist will have much to contribute here.

See also: **III / Pharmaceuticals:** Basic Drugs: Liquid Chromatography; Neutral and Acidic Drugs: Liquid Chromatography; Supercritical Fluid Chromatography; Thin-Layer (Planar) Chromatography. **III / Proteins:** High-Speed Countercurrent Chromatography.

Further Reading

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